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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT 2,4-Dichlorophenoxyacetic acid: Adverse Data Section 6(a) (2) Submission.

FROM: Jess Rowland, M.S, Toxicologist Jess Rowland 10/29/91
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and
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STUDY IDENTIFICATIONS: Case No: 818706 Submission No: 8402220

HED Project No.1-2254 Caswell No. 315

Registrant: Industry Task Force II Data on 2, 4-D Research Data.

ACTION REQUESTED: Review of subchronic feeding studies (MRID # 419915-01, mice and 419915-02, rats) for 2,4-Dichlorophenoxyacetic acid (2,4-D Acid). 6 (a) (2) data was submitted and reviewed by Tox.Branch for these guidelines on 6/30/90 .

RESPONSE: A separate Data Evaluation Report (DER) for each of the above referenced studies is attached. A summary of each study is as follows:

1. Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic acid. [MRID No. 419915].

SUMMARY: Male and female Fischer-344 rats were fed diets containing 2,4-Dichlorophenoxyacetic acid at 0, 1, 15, 100 or 300 mg/kg/day for 13 weeks.

Treatment did not cause any adverse effects at 1 or 15 mg/kg/day, but did cause decreases in mean body weight, body weight gain, alterations in some of the hematology and clinical chemistry parameters, changes in various organ weights, and histopathological changes in the liver, adrenals and kidneys at 100 mg/kg/day.

The highest dietary dose level of 300 mg/kg/day was associated with weight loss, alterations in hematology and clinical chemistry parameters, changes in various organ weights, and histopathological lesions in the eye, liver, testes, adrenals, kidneys, thymus, bone marrow, spleen, thyroid, and lungs.

Under the conditions of this study, a No-Observable-Effect Level (NOEL) of 15 mg/kg/day and a Lowest-Observable-Effect Level (LOEL) of 100 mg/kg/day are established. The LOEL is based on decreases in mean body weight and body weight gain, alterations in hematology and clinical chemistry parameters, and histopathological lesions observed at 100 mg/kg/day.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

2. Subchronic Toxicity Study in Mice with 2,4-Dichlorophenoxyacetic acid. [MRID No. 419915-02]

SUMMARY: Male and female B6C3F1 mice were fed diets containing 2,4-D at concentrations of 0, 1, 15, 100, or 300 mg/kg/day for 13 weeks. Treatment had no adverse effect on survival, body weight, body weight gain, food consumption, ophthalmoscopic examination, hematology and gross pathology at 1, 15, or 100 mg/kg/day dose levels.

Treatment-related changes at 100 mg/kg/day included decreased glucose (females) and decreased thyroxine (males) levels, increases in mean absolute and relative kidney weights (females), and a liver lesion in 1 female.

Treatment-related changes at 300 mg/kg/day included: transient decreases in food consumption (only up to Week 7); decreases in glucose (females) and thyroxine levels (males); significant decrease in kidney-to-brain weight ratios in males); correlating histopathological changes in the kidneys (karyomegaly, loss of brush order and decreased size of tubular lining cells) in males; and histopathological changes in the liver characterized by nuclear hyperchromatism, and decreased glycogen in periportal hepatocytes.

Under the conditions of this study, a NOEL of 15 mg/kg/day and a LOEL of 100.0 mg/kg/day is established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid to male and female mice. The LOEL is based on decreases in glucose and thyroxine levels, and increases in mean and relative kidney weight observed at 100 mg/kg/day.

CORE CLASSIFICATION: Not applicable since a subchronic study in mouse is not a Guideline requirement.

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Section II, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

TOX.CHEM. No.: 315 **MRID No.:** 419915-01

HED Project No. 1-2254 **Registrant:** Industry Task Force II

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid (2,4-D)

STUDY IDENTIFICATION: HLA Study No. 2184-116

TESTING LABORATORY: Hazleton Laboratories America, Rockville, Md.

TITLE OF REPORT: Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid.

REPORT AUTHOR: Gene E. Schulze, Ph.D., D.A.B.T

REPORT DATE: August 7, 1991

SUMMARY: Male and female Fischer-344 rats were fed diets containing 2,4-Dichlorophenoxyacetic acid at 0, 1, 15, 100 or 300 mg/kg/day for 13 weeks.

Treatment did not cause any adverse effects at 1 or 15 mg/kg/day, but did cause decreases in mean body weight, body weight gain, alterations in some of the hematology and clinical chemistry parameters, and changes in various organ weights, and histopathological changes in the liver, adrenals and kidneys at 100 mg/kg/day.

The highest dietary dose level of 300 mg/kg/day was associated with weight loss, alterations in hematology and clinical chemistry parameters, changes in various organ weights, and histopathological lesions in the eye, liver, testes, adrenals, kidneys, thymus, bone marrow, spleen, thyroid, and lungs.

Under the conditions of this study, a No-Observable-Effect Level (NOEL) of 15 mg/kg/day and a Lowest-Observable-Effect Level (LOEL) of 100 mg/kg/day are established. The LOEL is based on decreases in mean body weight, body weight gain and food consumption, alterations in hematology and clinical chemistry parameters, and histopathological lesions observed at 100 mg/kg/day.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of 2,4-Dichlorophenoxyacetic acid (2,4-D) following dietary administration to rats.

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: 2,4-dichlorophenoxyacetic acid.
Purity: 96.1%
Lot No.: 909
Description: Off-white powder.

2. Test Animals

Species: Rats
Strain: Fischer-344
Sex: Males and females
Age: Approximately 42 days at initiation.
Weight at initiation: 103.6-122.3 g (M); 84.4-104.2 g (F)
Identification: Tail tatoo and cage cards.
Acclimation: Approximately 2 weeks
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature, 72 ± 6°F and humidity 50 ± 20 %
Light/dark cycles: 12 hour photocycle.

3. Study Design

Group No.	Treatment	No. of Animals		Dose Level (mg/kg/day)
		Males	Females	
1	Control	10	10	0
2	Low	10	10	1
3	Mid-1	10	10	15
4	Mid-2	10	10	100
5	High	10	10	300

4. Test Article Formulation and Analyses

The test material was ground into fine powder with a mortar and pestle and a calculated amount for each dose level (adjusted to a purity of 100%) was weighed. A premix was prepared for each dose level with approximately 200 g of feed for approximately two minutes. The premixes of the respective dose levels were then added to the required amount of feed and mixed in a Hobart mixer for approximately 10 minutes.

Homogeneity was determined by a pretest mix analysis and stability tests were conducted prior to initiation at Days 0, 7 and 14. Control and test diets were analyzed for 2,4-D levels at Weeks 1, 2, 3, 4, 8 and 12.

5. Treatment

Rats were fed the control and test diets 7 days per week for at least 91 days. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. Cageside observations for clinical signs of toxicity were performed once daily. Body weights were obtained prior to initiation and once weekly thereafter. Food consumption was measured weekly. Physical examinations were performed and detailed clinical observations were recorded once per week at each weighing interval. Ophthalmologic examinations were performed on all rats once prior to initiation of dosing and prior to necropsy.

Blood and urine were collected from all animals at termination for hematology, clinical chemistry and urinalysis. The checked (x) parameters were determined.

Hematology

x Hematocrit (HCT)[a]	x Leukocyte count (WBC)[a]
x Hemoglobin (HGB)[a]	x Platelet count[a]
x Erythrocyte count (RBC)[a]	x Leukocyte differential[a]
Mean corpuscular HGB (MCH)	Mean corpuscular HGB Concentration (MCHC)
Mean corpuscular volume(MCV)	Erythrocyte fragility
x Corrected leukocyte count (COR WBC)	x Cell morphology

Clinical Chemistry

Electrolytes:	Other
x Calcium[a]	x Albumin[a]
x Chloride[a]	x Blood creatinine[a]
Magnesium[a]	x Blood urea nitrogen[a]
x Phosphorus[a]	Cholesterol[a]
x Potassium[a]	x Globulins
x Sodium	x Glucose[a]
	x Total bilirubin[a]
Enzymes:	x Total serum protein[a]
	Triglycerides
Alkaline phosphatase	Serum protein electrophoresis
x Alanine aminotransferase (SGPT)[a]	x Triiodothyronine (T_3)
	x Thyroxine (T_4)
x Aspartate aminotransferase (SGOT)[a]	
Cholinesterase[b]	
Creatinine phosphatase[a]	
Lactic acid dehydrogenase	
Gama glutamyl transferase	

Urinalysis

x Appearance[c]	x Bilirubin[c]
x Specific gravity[c]	x Occult blood[c]
x pH	x Urobilinogen
x Protein	x Glucose[c]
x Ketones[c]	Microscopic examination of sediment[c]

[a] Required for subchronic and chronic studies.
[b] Required only for organophosphates and carbamates.
[c] Required for chronic studies.

7. Termination

All surviving animals were fasted, weighed, anesthetized with sodium pentabarbital and exsanguinated. A gross necropsy was performed on Study Day 92 (males) and Study Day 93 (females), all gross pathological changes were recorded, and organs listed below were weighed.

Adrenals	Brain	Heart	Kidneys	Liver
Ovaries	Pituitary	Testes	Thyroids/parathyroids	Thymus

8. Histopathology

The checked (X) tissues from all rats were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Salivary glands[a]	Trachea[a]
x Esophagus[a]	x Lung[a]
x Duodenum[a]	Pharynx[a]
x Jejunum[a]	Larynx[a]
x Cecum[a]	Nose[a]
x Colon[a]	
x Rectum[a]	Cardiovascular/Hemo.System
x Liver[ac]	
x Pancreas[a]	x Aorta (thoracic)[a]
Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
Peripheral nerve[ab]	Urinogenital System
Spinal cord (3 levels)[ab]	
x Eyes (optical nerve)[ab]	x Kidneys[ac]
	x Urinary bladder[a]
Glandular System	x Testes[ac]
	x Epididymides
x Adrenals[a]	x Uterus[a]
Lacrimal glands[b]	x Ovaries[ac]
x Parathyroids[ad]	Others
x Thyroids[ad]	
	x All gross lesions and masses
	x Skeletal muscle[a]

a. Required for subchronic and chronic studies.
b. In subchronic studies examined only if indicated by toxicity or target organ involvement.
c. Organ weights required in subchronic and chronic studies.
d. Organ weights required for nonrodent studies.
e. Required for chronic inhalation study.

9. Statistical Analyses

In-life body weights, food consumption, hematologic and clinical chemistry parameters, and organ weight data were compared for differences of statistical significance from the same sex of treated groups. If variances of untransformed data were heterogeneous, analyses were performed on transformed data to achieve variance homogeneity. When the series of transformations were not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were performed routinely at the 5% two-tailed probability level.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Laboratories Inc, and the Study Protocol. A quality assurance statement was signed and dated August 6,1991.

II. RESULTS

1. Analysis of Diet Mix

Analytical results are presented in Table 1 (Appended). Routine concentration analyses showed that the mean concentration was within 10% of target level confirming accuracy of formulation. Homogeneity analyses indicated that the highest and lowest concentrations for Week 0 were homogeneous, and the stability analyses showed that the compound was stable in treated diet for at least a 14 day period.

2. Survival

Except for the accidental deaths of one male at 15 mg/kg/day and one female at 1 mg/kg/day during orbital sinus bleeding at Week 6, all animal survived to the end of the study.

3. Clinical Signs

Treatment-related clinical signs of toxicity observed in males and/or females at 300 mg/kg/day included few and/or no feces, pale and/or opaque eyes (also in females at 100 mg/kg/day), and depressed activity and hunched body posture (females only) . Other clinical signs occurred with low frequency sporadically throughout the groups.

4. Body Weights and Body Weight Changes

Mean body weights of both sexes of treated rats were lower than their respective controls during the study with the difference reaching statistical significance ($p < 0.05$) at Weeks 6 and 13. At Week 6, the decrease was 93 and 85% of control for males at 100 and 300 mg/kg/day, respectively, and 93 and 79% of controls for females at 100 and 300 mg/kg/day, respectively. This reduction can be atttributed to the overnight fast prior to scheduled bleeding. At week 13, the decrease was 92 and 77% of control for males at 100 and 300 mg/kg/day, respectively, and 94 and 72% of controls for males and females at 300 mg/kg/day, respectively.

Mean body weight change values are summarized in Tables 2 and 3 for males and females, respectively. Statistically significant ($p < 0.05$) reductions in body weight gain were observed in males at 300 mg/kg/day at Weeks 0 through 6 (72% of control) and in males at 100 and 300 mg/kg/day groups at Weeks 0 through 13 (91 and 63% of control, respectively). Significant ($p < 0.05$) decreases were seen in females at 100 and 300 mg/kg/day at Weeks 0 through 6 (83 and 50% of control, respectively) and in females at 300 mg/kg/day at Weeks 0 through 13 (43% of control). No reductions in body weight gain were seen in rats at 1 or 15 mg/kg/day groups.

Table 2. Mean Body Weight Change (G) in Male Rats

Dose (mg/kg/day)	0-6 Week	0-13 Week
0	136.5 ± 12.62	194.1 ± 18.51
1	133.7 ± 8.21	194.1 ± 10.69
15	129.2 ± 15.81	179.5 ± 21.89
100	124.7 ± 7.79	176.8 ± 10.25*
300	97.9 ± 11.76*	122.3 ± 19.47*

Table 3. Mean Body Weight Change (G) in Female Rats.

Dose (mg/kg/day)	0-6 Week	0-13 Week
0	58.5 ± 6.46	83.5 ± 8.11
1	57.7 ± 6.39	83.7 ± 8.24
15	63.0 ± 7.55	82.3 ± 7.23
100	48.7 ± 7.85*	73.9 ± 8.96
300	29.5 ± 5.73*	35.6 ± 13.16*

5. Food Consumption

No conclusions can be drawn from the food consumption data due to a number of confounding factors such as: an overnight fast prior to scheduled blood collections at Weeks 6 which led to statistically significant differences in mean food consumption values between created and control groups at Weeks 1 through 6; the decreased food consumption at 100 and 300 mg/kg/day groups due to non palatability of the diet; and the numerous instances of spilled feed at the highest dose level. Overall mean compound consumption (Weeks 1-13) was within 10% of target (mg/kg) values. The average daily compound intake during the study was 0.93, 13.98, 93.93, and 278.39 mg/kg/day for males and 0.96, 14.39, 96.16, and 293.42 mg/kg/day for females.

5. Ophthalmology Examination

Ophthalmoscopic findings are summarized in Tables 4 and 5 for males and females respectively. 2,4-D induced complete cataract formation in 7 females and posterior subcapsular cataract in 5 females at 300 mg/kg/day.

Table 4 Ophthalmoscopic Observations In Male Rats.

Observations	Dose Level (mg/kg/day)				
No.of animals = 10/dose level	0	1	15	100	300
Corneal Dystrophy	10	10	9	10	10
Retinal Linear Atrophy	1	1	1	0	0
Retinal Degeneration	0	1	1	1	0
Chromodacryorhhea	0	0	0	0	0
Complete Cataract	0	0	0	1	0
Posterior Subcapsular Cataract	0	0	0	0	0
Phthisis Bulbi	0	0	0	1	0
Corneal Ulcer	0	0	0	0	1

Table 5 Ophthalmoscopic Observations In Female Rats.

Observations	Dose Level (mg/kg/day)				
No.of animals = 10/dose level	0	1	15	100	300
Corneal Dystrophy	10	9	10	10	10
Retinal Linear Atrophy	9	9	10	10	9
Retinal Degeneration	2	2	0	1	1
Chromodacryorhhea	0	0	1	1	0
Complete Cataract	0	0	0	1	7
Posterior Subcapsular Cataract	0	0	0	0	5
Phthisis Bulbi	0	0	0	0	0
Corneal Ulcer	0	0	0	0	0

6. Hematology and Clinical Chemistry

Treatment-related, statistically significant ($p \leq 0.05$) increases (↑) or decreases (↓) observed in hematology parameters are summarized below:

Hematology

Parameter	Males				Females			
	100 mg/kg/day		300 mg/kg/day		100 mg/kg/day		300 mg/kg/day	
Week	6	13	6	13	6	13	6	13
RBC	↑	-	-	↓	-	-	↓	↓
HGB	↑	-	-	↓	-	-	↓	↓
HCT	↑	-	-	-	-	-	↓	↓
Platelet	-	↓	↓	↓	-	↓	↓	↓
WBC	-	-	↓	↓	-	-	↓	↓
COR WBC	-	-	↓	↓	-	-	↓	↓
LYMPH	-	-	↓	-	-	-	↓	-
SEG	-	-	-	-	-	-	↓	-

Clinical Chemistry

Parameter	Males				Females			
	100 mg/kg/day		300 mg/kg/day		100 mg/kg/day		300 mg/kg/day	
Week	6	13	6	13	6	13	6	13
Glucose	-	↓	-	↓	-	↓	-	-
CREAT	-	-	-	-	↑	↑	↑	-
ALT	-	↑	-	↓	↑	-	↑	-
T PROT	↓	↓	↓	-	-	-	-	-
Globulin	-	-	-	↓	-	-	-	↓
Calcium	↓	-	-	-	-	-	-	-
T3	-	-	↓	↓	↓	↓	-	↓
T4	↓	↓	↓	↓	↓	↓	↓	↓

7. Gross Pathology

Treatment-related gross necropsy findings included: various eye lesions (primarily in females at 300 mg/kg/day); pale adrenal (males and females at 300 mg/kg/day); pale lung (females at 300 mg/kg/day); pale and/or prominent reticular pattern in liver (males and females at 300 mg/kg/day); and small testes and epididymides (males at 300 mg/kg/day).

8. Organ Weights

Treatment-related, statistically significant ($p \leq 0.05$) increases (↑) or decreases (↓) in absolute and relative organ weight primarily seen in male and/or female rats at 100 and 300 mg/kg/day are tabulated below:

Changes in Absolute and Relative Organ Weights in Male Rats

	Males			
	100 mg/kg/day		300 mg/kg/day	
Oragan	Absolute	Relative	Absolute	Relative
Adrenal	-	↑	-	↑
Brain	-	↑	-	↑
Heart	↓	-	↓	↑
Kidney	↑	↑	↓	↑
Liver	-	↑	-	↑
Pituitary	-	-	-	↑
Testes	-	-	↓	↓
Thymus	-	-	↓	-
Thyroid	-	↑	↑	↑

Changes in Absolute and Relative Organ Weights in Female Rats

	Females			
	100 mg/kg/day		300 mg/kg/day	
Oragan	Absolute	Relative	Absolute	Relative
Adrenal	-	↑	↓	-
Brain	-	↑	↓	↑
Heart	-	-	↓	↑
Kidney	-	↑	↓	↑
Liver	-	↑	-	↑
Pituitary	-	-	↓	↓
Ovary	-	-	↓	↓
Thymus	-	-	↓	↓
Thyroid	-	↑	-	↑

9. Histopathology

Treatment-related histopathological changes (Tables 6 and 7) observed at 100 and 300 mg/kg/day included:

1. Bilateral cataract formation (cataractous change) and retinal degeneration in females at 300 mg/kg/day.

2. Centrilobular hepatocellular hypertrophy of the liver in males at 100 and 300 mg/kg/day and in females at 300 mg/kg/day.

3. Atrophy of the testes in males at 300 mg/kg/day.

4. Hypertrophy of the zona glomerulose of the adrenal cortex in males and females at 100 and 300 mg/kg/day.

5. Brush border loss in proximal tubular cells of kidneys in males at 100 and 300 mg/kg/day and in females at 300 mg/kg/day.

6. Atrophy of the thymus in females at 300 mg/kg/day.,

7. Alveolar macrophage accumulation of the lung in males and females at 300 mg/kg/day.

8. Hypocellularity of bone marrow in males and females at 300 mg/kg/day.

9. Atrophy of the spleen in males and females at 300 mg/kg/day.

10. Follicular cell hyperplasia of thyroid in females at 300 mg/kg/day.

Histopathological evaluation of the eye revealed cataractous changes (cataracts) in the lens which were moderately severe in 4 rats and severe in 3 rats at 300 mg/kg/day. Among females at 100 mg/kg/day, this finding was moderate in 1, minimal in another, but unilateral in both. Bilateral retinal degeneration observed only in 2 females at 300 mg/kg/day.

In some cases, the histopathological changes correlated well with alterations observed in hematology and clinical chemistry parameters and organ weight data. Increase in liver weight, alanine aminotransferase, and aspartate aminotransferase were associated with centrilobular hepatocellular hypertrophy of the liver. Decreased thyroxine levels can be correlated with follicular cell hypertrophy of the thyroid gland. Increase in adrenal weight can be correlated with hypertrophy of cells of the zona glomerulose. Decreased mean thymic weight can be histologically correlated with atrophy observed in both sexes at the high dose. Atrophy of mesenteric adipose tissue in the peritoneal cavity can be correlated with mean body weight decrease in males and females at the high dose. Other microscopical changes were considered incidental and unrelated to treatment.

Table 7. Histopathological Findings in Male Rats At Sacrifice.

Organ / Lesion	Dose Level (mg/kg/day)				
No.of animals= 10/dose levels	0	1	15	100	300
Adrenal, cortex -Hypertrophy, zona glomerulose	0	0	0	1	8
Thyroid -Hypertrophy	0	0	0	0	0
Liver -Hypertrophy, centrilobular	0	0	0	3	7
Lung -Alveolar macrophage accumulation	0	0	0	0	5
Spleen -Atrophy	0	0	0	0	10
Kidney -Brush border loss, tubular	0	0	0	0	6
Testes -Atrophy, bilateral	0	0	0	0	8
Thymus -Atrophy	0	0	0	0	4
Eye -Cataractous change, unilateral	0	0	0	1	0

Table 8. Histopathological Findings in Female Rats At Sacrifice.

Organ / Lesion	Dose Level (mg/kg/day)				
No.of animals= 10/dose levels	0	1	15	100	300
Adrenal, cortex -Hypertrophy, zona glomerulose	0	0	0	10	10
Thyroid -Hypertrophy	3	0	0	0	8
Liver -Hypertrophy, centrilobular	0	0	0	0	9
Lung -Alveolar macrophage accumulation	0	0	0	0	10
Spleen -Atrophy	0	0	0	0	10
Kidney -Brush border loss, tubular	0	0	0	1	10
Thymus -Atrophy	0	0	0	0	8
Eye -Cataractous change, bilateral -Cataractous change, unilateral -Degeneration, retinal, bilateral	 0 0 0	 0 0 0	 0 0 0	 0 2 0	 5 2 2

IV. DISCUSSION

Male and female Fischer-344 rats were fed diets containing 2,4-D at concentrations of 0, 1, 15, 100, or 300 mg/kg/day for 13 weeks.

Analytical data showed that the diet mixes were homogeneous, stable at room temperature for up to 14 days and the mean concentrations were within 10% of the target level.

Treatment had no adverse effect on survival. No treatment-related effects were seen at 1 mg/kg/day or 15 mg/kg/day dose levels.

Treatment-related clinical signs were observed at the high-dose included few or no feces, pale and/or opaque eyes (also in males at 100 mg/kg/day) depressed activity and hunched body posture (female at the high-dose only).

Decreases in mean body weight and body weight gain were observed in males at 100 mg/kg/day and in both sexes at 300 mg/kg/day.

Ophthalmology examinations revealed bilateral cataract formation only in females at 300 mg/kg/day; no ocular changes were seen in male at the high dose or in either sex at the lower doses.

Treatment-related alterations in hematology parameters were confined primarily to the high dose included: decreased red cell mass (erythrocyte, counts, and hemoglobin), decreased white cell and lymphocyte count values, decreased platelet counts (also in males and females at 100 mg/kg/day) and decreased incidence/grade for echinocytes.

Also, treatment-related alterations in clinical chemistry parameters were primarily seen at 100 and 300 mg/kg/day groups which included: decreased glucose, globulin, and thyroxine levels, and decreased triiodothyronine levels. No biologically important changes were seen from urinalysis.

Treatment-related gross pathological changes were limited to the high dose group and included small and soft testes, opaque eyes, pale adrenals, pale areas of lung, and pale and/or prominent reticular pattern in the liver.

Treatment-related changes were observed in organ weight data at 100 and 300 mg/kg/day and included decreased mean relative and absolute ovary weights, increased relative thyroid weights, decreased relative and absolute testes with epididymides weights, increased absolute kidney weight, increased relative adrenal weights, and increased relative liver weights.

In most cases, alterations noted in hematology, clinical chemistry, gross pathology, and organ weights correlated well with histopathological changes. Treatment-related histopathological changes which were limited to 100 and 300 mg/kg/day group and included bilateral cataract formation and retinal degeneration (females at high dose only), centrilobular hepatocellular hypertrophy, atrophy of the testes, hypertrophy in the zona glomerulose of the adrenal cortex, brush order loss in proximal tubular cells in the kidneys, atrophy of the thymus, hypocellularity of bone marrow, splenic atrophy, follicular cell hypertrophy of the thyroid, and alveolar macrophage accumulation of the lung.

V. CONCLUSION

Under the conditions of this study, a NOEL of 15 mg/kg/day and a LOEL of 100.0 mg/kg/day is established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid to male and female rats. The LOEL is based on decreases in mean body weight, body weight gain, alterations in hematology and clinical chemistry parameter, changes in organ weights, and histopathological changes observed at 100 mg/kg/day.

VI. **CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

APPENDIX

Table 1
Analytical Chemistry Resul's
Subchronic Toxicity Study in Rats with
2,4-Dichlorophenoxyacetic Acid

Table 1
Results of Test Diet Analyses
Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid

Homogeneity (Pretest mix)

	Target Concentration (ppm)				Assayed Level (ppm)			Percent Target		
	0	8	6195		0	8	6195	0	8	6195
	0	-	-		ND	-	-	-	-	-
Top	-	8	6195	A	-	7.182	6514	-	89.8	105
	-	8	6195	B	-	6.897	6550	-	86.2	106
Middle	-	8	6195	A	-	7.461	6352	-	93.3	103
	-	8	6195	B	-	7.461	6045	-	93.3	97.6
Bottom	-	8	6195	A	-	7.461	6438	-	93.3	104
	-	8	6195	B	-	7.182	6201	-	89.8	100
								Mean	91.8	103
								S.D.	2.89	3.20
								% RSD	3.2	3.1

NOTES: A and B are duplicate samples.
ND = Not detected.

Table 1 - Continued
Results of Test Diet Analyses
Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid

Pretest Stability

	Target Concentration (ppm)				Assayed Level (ppm)			Percent Target		
	0	8	6195		0	8	6195	0	8	6195
Day 0[a]	0	-	-		-	-	-	-	-	-
	0	8	6195	A	ND	7.368	-	-	92.13	104
	-	8	6195	B	ND	7.180	-	-	89.77	101
Day 7	0	-	-		-	-	-	-	-	-
	0	8.00	6195	A	ND	8.500	6272	-	106	101
	-	8.00	6195	B	ND	8.750	6272	-	109	101
Day 14	0	-	-		-	-	-	-	-	-
	0	8.00	6195	A	ND	8.883	5944	-	111	96.0
	-	8.00	6195	B	ND	7.786	6156	-	97.3	99.4

NOTES: A and B are duplicate samples.
ND = Not detected.

[a] Mean of homogeneity mix.

Table 1 - Continued
Results of Test Diet Analyses
Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid

Males

Group:	Target Concentration (ppm)					Assayed Level (ppm)				Percent Target			
Dose Level (mg/kg/day):	2 1	3 15	4 100	5 300		2 1	3 15	4 100	5 300	2 1	3 15	4 100	5 300
Week													
1	8.2899	125.3589	888.0947	2545.8632	A	7.747	124.2	812.0	2481	94.4	99.1	108	97.5
					B	8.289	124.2	819.1	2481	101	99.1	101	97.5
2	9.6899	146.9174	978.4731	2545.8632	A	9.396[a]	147.5	1025	2595	97.0	100	105	102
					B	10.18[a]	147.5	991.3	2595	105	100	101	102
3	10.5645	159.4836	1096.4216	3415.7568	A	11.19	169.3	1077	3695	106	106	98.4	108
					B	10.67	158.8	1118	3533	101	99.6	102	103
4	11.9388	188.7356	1197.3313	3784.8643	A	13.09	198.9	1279[a]	3692	110	110	107	99.7
					B	12.81	186.9	1317[a]	3376	107	103	110	91.1
8	15.2544	235.8272	1540.1973	4549.9297	A	14.24[a]	244.7	1595	4571	93.5	104	104	100
					B	13.84[a]	226.1	1536	4807	90.8	95.9	99.7	106
12	18.3899	278.0094	1726.9937	5259.3750	A	19.34[a]	262.2	1703	5281	105	94.3	98.6	100
					B	17.74[a]	262.2	1589	5427	96.5	94.3	92.0	103

NOTES: Group 1 basal diet was analyzed for all weeks with no compound detected.
A and B are duplicate samples.

[a] Results from re-extraction.

Table 1 - Continued
Results of Test Diet Analyses
Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid

Females

Group:	Target Concentration (ppm)					Assayed Level (ppm)				Percent Target			
Dose Level (mg/kg/day):	2 1	3 15	4 100	5 300		2 1	3 15	4 100	5 300	2 1	3 15	4 100	5 300
Week													
1	9.5500	142.9950	960.5000	2888.6000	A	9.571	144.2	941.9	2717	100	101	98.1	96.7
					B	9.317	137.6	970.7	2717	97.6	96.2	101	96.7
2	8.9249	131.5401	901.8285	2888.6000	A	9.337	128.5	1025	2940	105	97.7	104	105
					B	9.337	132.3	1025	2768	105	101	104	98.6
3	9.4300	144.6328	994.0966	3036.9565	A	9.861[a]	151.9	974.0	2964	105	105	98.0	97.6
					B	10.36[a]	151.9	1036	3046	110	105	104	100
4	9.8154	150.9036	1052.6824	3151.8462	A	10.25	162.8[a]	1025	3165	104	108	97.4	100
					B	9.962	166.7[a]	1052	3165	101	110	99.9	100
8	11.8967	182.1635	1292.8747	3789.0547	A	10.87[a]	177.2	1355	3750	91.4	97.3	105	99
					B	10.87[a]	194.5	1338	3892	91.4	107	104	103
12	14.3254	223.8384	1384.8313	4321.6943	A	14.06[a]	201.5[a]	1292	4317	98.1	90.0	93.3	99.9
					B	13.79[a]	223.2[a]	1399	4048	96.3	99.7	101	93.7

NOTES: Group 1 basal diet was analyzed for all weeks with no compound detected.
A and B are duplicate samples.

[a] Results from re-extraction.

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PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist Jess Rowland 9/29/91
Section II, Toxicology Branch II (H7509C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 10/29/91
Section II, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

TOX.CHEM. No.: 315 **MRID No.:** 419915-02

HED Project No. 1-2254 **Registrant:** Industry Task Force II

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid (2,4-D)

STUDY IDENTIFICATION: HLA Study No. 2184-117

TESTING LABORATORY: Hazleton Laboratories America, Rockville, Md.

TITLE OF REPORT: Subchronic Toxicity Study in Mice with 2,4-Dichlorophenoxyacetic Acid.

REPORT AUTHOR: Gene E. Schulze, Ph.D., D.A.B.T

REPORT DATE: August 16, 1991

SUMMARY: Male and female B6C3F1 mice were fed diets containing 2,4-D at concentrations of 0, 1, 15, 100, or 300 mg/kg/day for 13 weeks. Treatment had no adverse effect on survival, body weight, body weight gain, food consumption, ophthalmoscopic examination, hematology and gross pathology at 1, 15, or 100 mg/kg/day dose levels. Treatment-related changes at 100 mg/kg/day included decreased glucose (females) and decreased thyroxine (males) levels, increases in mean absolute and relative kidney weights (females), and a liver lesion in 1 female. Treatment-related changes at 300 mg/kg/day included: transient decreases in food consumption (only up to Week 7); decreases in glucose (females) and thyroxine levels (males); significant decrease in kidney-to-brain weight ratios in males); correlating histopathological changes in the kidneys (karyomegaly, loss of brush order and decreased size of tubular lining cells) in males; and histopathological changes in the liver characterized by nuclear hyperchromatism, and decreased glycogen in periportal hepatocytes. Under the conditions of this study, a NOEL of 15 mg/kg/day and a LOEL of 100.0 mg/kg/day is established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid to male and female mice. The LOEL is based on decreases in glucose and thyroxine levels, and increases in mean and relative kidney weight observed at 100 mg/kg/day.

VI. **CORE CLASSIFICATION:** Not applicable; a 90-day study in mice is not a guideline requirement.

I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of 2,4-Dichlorophenoxyacetic acid (2,4-D) following dietary administration to mice.

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: 2,4-dichlorophenoxyacetic acid.
Purity: 96.1%
Lot No.: 909
Description: Off-white powder.

2. Test Animals

Species: Mice
Strain: B6C3F1
Sex: Males and females
Age: Approximately 43 days at initiation.
Weight at initiation: 20.6-23.9 g (M); 17-20.2 g (F)
Identification: Ear tag and cage cards.
Acclimation: Approximately 2 weeks
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature, 72 ± 6°F and humidity 50 ± 20 %
Light/dark cycles: 12 hour photocycle.

3. Study Design

Group No.	Treatment	No. of Animals		Dose Level (mg/kg/day)
		Males	Females	
1	Control	10	10	0
2	Low	10	10	1
3	Mid-1	10	10	15
4	Mid-2	10	10	100
5	High	10	10	300

4. Test Article Formulation and Analyses

The test material was ground into fine powder with a mortar and pestle and a calculated amount for each dose level (adjusted to a purity of 100%) was weighed. A premix was prepared for each dose level with approximately 200 g of feed for approximately 2 minutes. The premixes of the respective dose levels were then added to the required amount of feed and mixed in a Patterson-Kelly twin shell blender for approximately 10 minutes.

Homogeneity was determined by a pretest mix analysis and stability tests were conducted prior to initiation at Days 0, 7 and 14. Additional homogeneity analysis was performed at Week 1 due to a change in batch size of the dietary mixes. Control and test diets were analyzed for 2,4-D levels at Weeks 1, 2, 3, 4, 8 and 12.

5. Treatment

Mice were fed the control and test diets 7 days per week for at least 91 days. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. Cageside observations for clinical signs of toxicity were performed once daily. Body weights were obtained prior to initiation and once weekly thereafter. Food consumption was measured weekly. Physical examinations were performed and detailed clinical observations were recorded once per week at each weighing interval. Ophthalmologic examinations were performed on all mice once prior to initiation of dosing and prior to necropsy.

Blood and urine were collected from all animals at termination for hematology, clinical chemistry and urinalysis. The checked (x) parameters were determined.

Hematology

x Hematocrit (HCT)[a]	x Leukocyte count (WBC)[a]
x Hemoglobin (HGB)[a]	x Platelet count[a]
x Erythrocyte count (RBC)[a]	x Leukocyte differential[a]
Mean corpuscular HGB (MCH)	Mean corpuscular HGB Concentration (MCHC)
Mean corpuscular volume(MCV)	Erythrocyte fragility
x Corrected leukocyte count (COR WBC)	x Cell morphology

Clinical Chemistry

Electrolytes:	Other
x Calcium[a] x Chloride[a] Magnesium[a] x Phosphorus[a] x Potassium[a] x Sodium	x Albumin[a] x Blood creatinine[a] x Blood urea nitrogen[a] Cholesterol[a] x Globulins x Glucose[a] x Total bilirubin[a] x Total serum protein[a] Triglycerides Serum protein electrophoresis Triiodothyronine (T_3) x Thyroxine (T_4)
Enzymes:	
Alkaline phosphatase x Alanine aminotransferase (SGPT)[a] x Aspartate aminotransferase (SGOT)[a] Cholinesterase[b] Creatinine phosphatase[a] Lactic acid dehydrogenase Gama glutamyl transferase	

[a] Required for subchronic and chronic studies.
[b] Required only for organophosphates and carbamates.

7. Termination

All surviving animals were fasted, weighed, anesthetized with sodium pentobarbital and exsanguinated. A gross necropsy was performed on Study Day 91 and all gross pathological changes were recorded, and organs listed below were weighed.

Adrenals	Brain	Heart	Kidneys	Liver
Ovaries	Pituitary	Testes	Thyroids/parathyroids	Thymus

8. Histopathology

The checked (X) tissues from all animals were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Salivary glands[a]	x Trachea[a]
x Esophagus[a]	x Lung[a]
x Duodenum[a]	Pharynx[a]
x Jejunum[a]	Larynx[a]
x Cecum[a]	Nose[a]
x Colon[a]	
x Rectum[a]	Cardiovascular/Hemo.System
x Liver[ac]	
x Pancreas[a]	x Aorta (thoracic)[a]
x Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
Peripheral nerve[ab]	Urinogenital System
Spinal cord (3 levels)[ab]	
x Eyes (optical nerve)[ab]	x Kidneys[ac]
	x Urinary bladder[a]
Glandular System	x Testes[ac]
	x Epididymides
x Adrenals[a]	x Uterus[a]
Lacrimal glands[b]	x Ovaries[ac]
x Parathyroids[ad]	Others
x Thyroids[ad]	
	x All gross lesions and masses
	Skeletal muscle[a]

a. Required for subchronic and chronic studies.
b. In subchronic studies examined only if indicated by toxicity or target organ involvement.
c. Organ weights required in subchronic and chronic studies.
d. Organ weights required for nonrodent studies.
e. Required for chronic inhalation study.

9. Statistical Analyses

In-life body weights, food consumption, hematologic and clinical chemistry parameters, and organ weight data were compared for differences of statistical significance from the same sex of treated groups. If variances of untransformed data were heterogeneous, analyses were performed on transformed data to achieve variance homogeneity. When the series of transformations were not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were performed routinely at the 5% two-tailed probability level.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Laboratories Inc, and the Study Protocol. A quality assurance statement was signed and dated August 16,1991.

II. RESULTS

1. Analysis of Diet Mix

Routine concentration analyses showed that the mean concentration was within 11% of target level confirming accuracy of formulation. Homogeneity analyses indicated that the highest and lowest concentrations for Week 0 were homogeneous, and the stability analyses showed that the compound was stable in treated diet for at least a 14 day period.

2. Survival

Except for the accidental death of one male at 100 mg/kg/day and the death of one female in the control group, all animal survived to the end of the study.

3. Clinical Signs

No treatment-related clinical signs of toxicity were observed at any dose level. Clinical signs such as alopecia, hunched posture, sores, swollen abdomen and few or no feces were seen in both the control and treated groups.

4. Body Weights and Body Weight Changes

Treatment had no effect on mean body weight or body weight gain. Both sexes of mice gained weight during the study and the mean body weight gain among the treated animals were similar to that of the weight gain seen in the control mice.

5. Food Consumption

Consistent decreases in mean food consumption were noted in males and female at 300 mg/kg/day group during the first 7 weeks of treatment, after which food consumption values were comparable to controls. The decreases reached statistical significance ($p < 0.05$) only during study Weeks 2, 3, and 6 for males and Weeks 1,2,4, and 7 for females. Significant decreases were also noted for males at 100 mg/kg/day at Week 3 and 7.

Overall mean compound consumption (Weeks 1-13) was within 10% of target (mg/kg) values. The average daily compound intake during the study was 0.98, 14.71, 98.2, and 292.61 mg/kg/day for males and 0.99, 14.84, 98.85, and 295.88 mg/kg/day for females.

5. Ophthalmology Examination

No treatment-related ophthalmoscopic findings were seen.

6. Hematology and Clinical Chemistry

Significant changes in hematology parameters were decreased leukocyte, corrected leukocyte, and lymphocyte counts in males at 15, 100 and 300 mg/kg/day. Although these decreases were statistically significant ($p < 0.05$), they were not considered to be treatment related due to (i) abnormally high leukocyte and lymphocyte counts in control males (mean 4.1 ± 1.21 TH/UL) as compared to the control females (mean 2.1 ± 0.7 TH/UL), (ii) the lack of consistency between sexes, and (iii) the lack of clear dose-response. In females at the high dose, the erythrocyte count was slightly, but significantly decreased (10.26 MI/UL vs. 10.58 MI/UL in controls).

Clinical chemistry parameters in treated males were comparable to control males. The glucose level was significantly ($p < 0.05$) decreased in females at 1, 100 and 300 mg/kg/day levels. Thyroxine levels < 2 µg/DL were seen in all males at 100 and 300 mg/kg/day suggesting a potential for a hypothyroid state in this sex; however, the statistical interpretation is somewhat confounded by the limited number of available samples. In females, decreases in thyroxine values occurred in 1 animal at 100 mg/kg/day and in 2 at 300 mg/kg/day.

7. Gross Pathology

No treatment-related gross necropsy findings were seen at any dose level.

8. Organ Weights

Significant, treatment-related changes in organ weights included: increased mean absolute kidney weights (females at 100 mg/kg/day); increased relative kidney weights (females at 100 and 300 mg/kg/day); and decreased kidney-to-brain-weights in males at 300 mg/kg/day. However, the biological significance of the changes in the kidney weights is not clear due to lack of histological correlation in the kidneys of females and the lack of a clear dose-response. Other statistically significant changes in organ weight data were not considered to be treatment-related due to a lack of dose response, a lack of consistency between sexes, and no correlation to clinical pathology data or histopathology.

9. Histopathology

Treatment-related histopathological changes were limited to the kidneys of males at 300 mg/kg/day and in the liver of males and females at 300 mg/kg/day.

Kidney lesions were characterized by decrease in size of tubular lining cells in the proximal tubules, occasional karyomegaly, and loss of brush order. These findings were noted in 9/10 males only at the high dose; females were unaffected.

Liver lesions were characterized by dark staining and densely clumped chromatin (nuclear hyperchromatism) seen in 8/10 males and 8/10 female at 300 mg/kg/day. In addition, hepatocytes in the periportal region were smaller due to decreased glycogen. This findings was also present in 1/10 females at 100 mg/kg/day.

IV. DISCUSSION

Male and female B6C3F1 mice were fed diets containing 2,4-D at concentrations of 0, 1, 15, 100, or 300 mg/kg/day for 13 weeks.

Analytical data showed that the diet mixes were homogeneous, stable at room temperature for up to 14 days and the mean concentrations were within 11% of the target level.

Treatment had no adverse effect on survival, body weight, body weight gain, food consumption, ophthalmoscopic examination, hematology and gross pathology at 1, 15, or 100 mg/kg/day dose levels.

Treatment-related changes at 100 mg/kg/day included decreased glucose (females) and decreased thyroxine (males) levels, increases in mean absolute and relative kidney weights (females), and a liver lesion in 1 female.

Treatment-related changes at 300 mg/kg/day included: transient decreases in food consumption (only up to Week 7); decreases in glucose (females) and thyroxine levels (males); significant decrease in kidney-to-brain weight ratios in males); correlating histopathological changes in the kidneys (karyomegaly, loss of brush order and decreased size of tubular lining cells) in males; and histopathological changes in the liver characterized by nuclear hyperchromatism, and decreased glycogen in periportal hepatocytes.

V. CONCLUSION

Under the conditions of this study, a NOEL of 15 mg/kg/day and a LOEL of 100.0 mg/kg/day is established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid to male and female mice. The LOEL is based on decreases in glucose and thyroxine levels, and increases in mean and relative kidney weight observed at 100 mg/kg/day.

VI. **CORE CLASSIFICATION:** Not applicable; a 90-day study in mice is not a guideline requirement.

Table 1 - Continued
Results of Test Diet Analyses
Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid

Females

Dose Level (mg/kg/day):	Group:	Target Concentration (ppm) 2 / 1	3 / 15	4 / 100	5 / 300		Assayed Level (ppm) 2 / 1	3 / 15	4 / 100	5 / 300	Percent Target 2 / 1	3 / 15	4 / 100	5 / 300
Week														
1		9.5500	142.9950	960.5800	2888.6800	A	9.571	144.2	961.9	2717	100	101	98.1	96.7
						B	9.317	137.6	970.7	2717	97.6	96.2	101	96.7
2		8.9249	131.5401	981.8285	2888.6800	A	9.337	128.5	1025	2940	105	97.7	104	105
						B	9.337	132.3	1025	2768	105	101	104	98.6
3		9.4308	144.6328	994.0966	3036.9565	A	9.861[a]	151.9	974.0	2964	105	105	98.0	97.6
						B	10.36[a]	151.9	1036	3046	110	105	104	100
4		9.8154	150.9036	1052.6824	3151.8462	A	10.25	162.0[a]	1025	3165	104	108	97.4	100
						B	9.962	166.7[a]	1052	3165	101	110	99.9	100
8		11.8967	182.1635	1292.0747	3789.0547	A	10.87[a]	177.2	1355	3750	91.4	97.3	105	99
						B	10.87[a]	194.5	1338	3892	91.4	107	104	103
12		14.3254	223.8384	1384.8513	4321.6943	A	14.06[a]	201.5[a]	1292	4317	98.1	90.0	93.3	99.9
						B	13.79[a]	223.2[a]	1399	4048	96.3	99.7	101	93.7

NOTES: Group 1 basal diet was analyzed for all weeks with no compound detected.
A and B are duplicate samples.

[a] Results from re-extraction.

Table 1 - Continued
Results of Test Diet Analyses
Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid

Males

Dose Level (mg/kg/day):	Target Concentration (ppm)					Assayed Level (ppm)				Percent Target			
Group:	2	3	4	5		2	3	4	5	2	3	4	5
	1	15	100	300		1	15	100	300	1	15	100	300
Week													
1	8.2099	125.3589	808.0947	2545.8632	A	7.747	124.2	812.8	2481	94.4	99.1	100	97.5
					B	8.289	124.2	819.1	2481	101	99.1	101	97.5
2	9.6899	146.9174	978.4731	2545.8632	A	9.398[a]	147.5	1025	2595	97.0	100	105	102
					B	10.18[a]	147.5	991.3	2595	105	100	101	102
3	10.5645	159.4836	1096.4216	3415.7568	A	11.19	169.3	1077	3495	106	106	98.4	108
					B	10.67	158.8	1118	3533	101	99.6	102	103
4	11.9388	180.7356	1197.3313	3704.8643	A	13.09	198.9	1279[a]	3692	110	110	107	99.7
					B	12.81	186.9	1317[a]	3376	107	103	110	91.1
8	15.2344	235.8272	1540.1973	4549.9297	A	14.24[a]	244.7	1595	4571	93.5	104	104	100
					B	13.84[a]	226.1	1536	4807	90.8	95.9	99.7	106
12	18.3899	278.0094	1726.9937	5259.3750	A	19.34[a]	262.2	1783	5281	105	94.3	98.6	100
					B	17.74[a]	262.2	1589	5427	96.5	94.3	92.0	103

NOTES: Group 1 basal diet was analyzed for all weeks with no compound detected.
A and B are duplicate samples.

[a] Results from re-extraction.

END